

APPENDIX – FIGURES

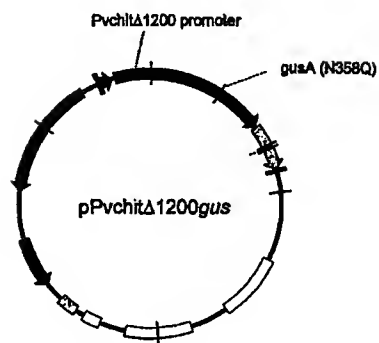


Figure 1. Schematic representation of the binary plasmid pPvchitΔ1200gus, used as vector for the reporter gene GUS under the control of part (-1200) of the bean chitinase promoter.



Figure 2 – Photograph of a tobacco flower, clearly showing the anthers.

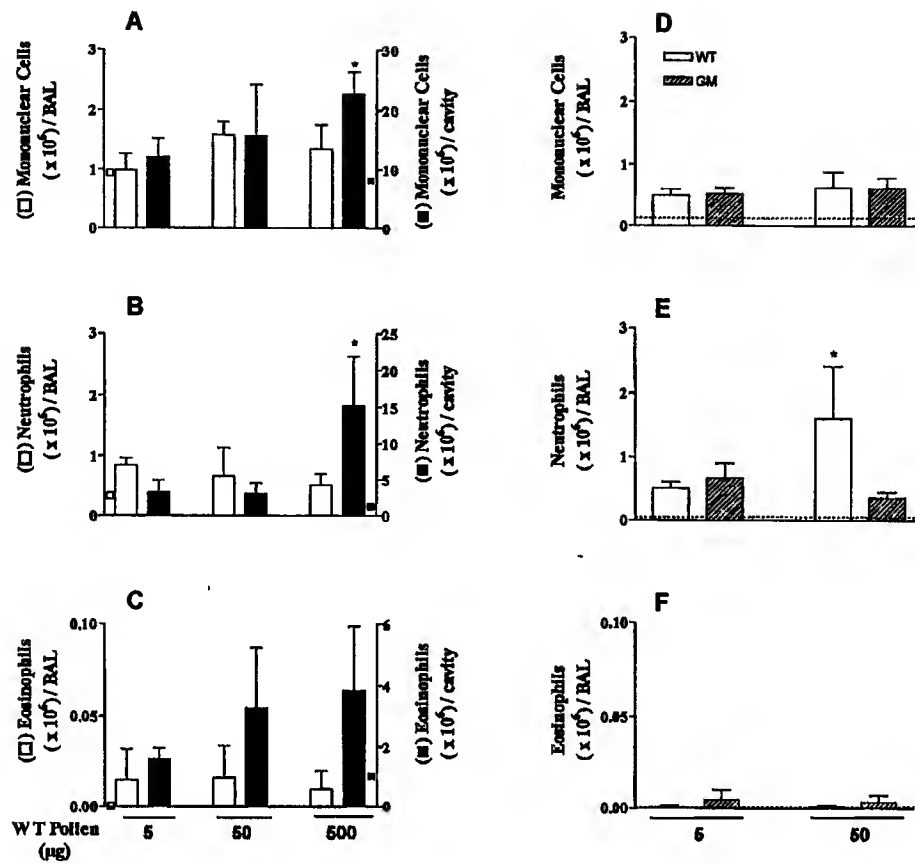


Figure 3 – Counts of mononuclear cells A), neutrophils B), and eosinophils C), in alveolar lavage (BAL) of rats subjected to three consecutive instillations of pollen grains in the indicated concentrations and in pleural lavage of rats subjected to intrapleural injections of pollen grains in the same concentrations. □ and ■ represent, respectively, the average values of BAL and pleural controls. C), D), and F) represent, respectively, the counts of mononuclear cells, neutrophils and eosinophils of broncho-alveolar lavage of rats subjected to three consecutive instillations of WT or GM pollen grains in the indicated concentrations. Dashed lines represent the average values of the controls instilled with saline solution. Columns represent averages \pm E. P. M. and asterisks indicate statistically significant differences ($p < 0.05$).

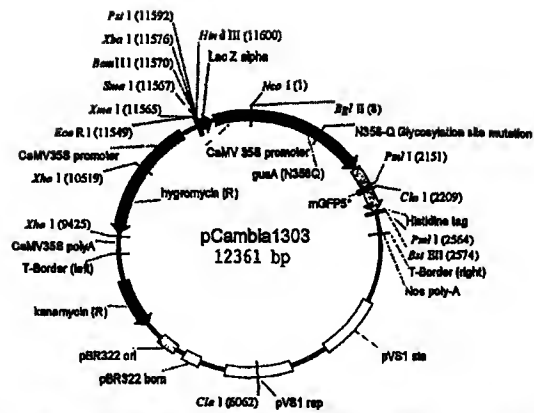


Figure 4. Schematic representation of binary plasmid pCambia 1303, used as vector having the reporter genes GFP, GUS and of the selection markers for Kanamycin and hygromycin. Single restriction sites *Xba*I and *Nco*I and other single and double restriction sites are also indicated.

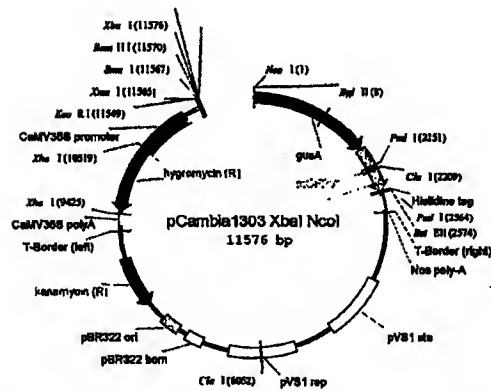


Figure 5. Schematic representation of the binary plasmid pCambia1303 without the CaMV 35S promoter region, as per the cleavage with *Xba*I and *Nco*I enzymes.

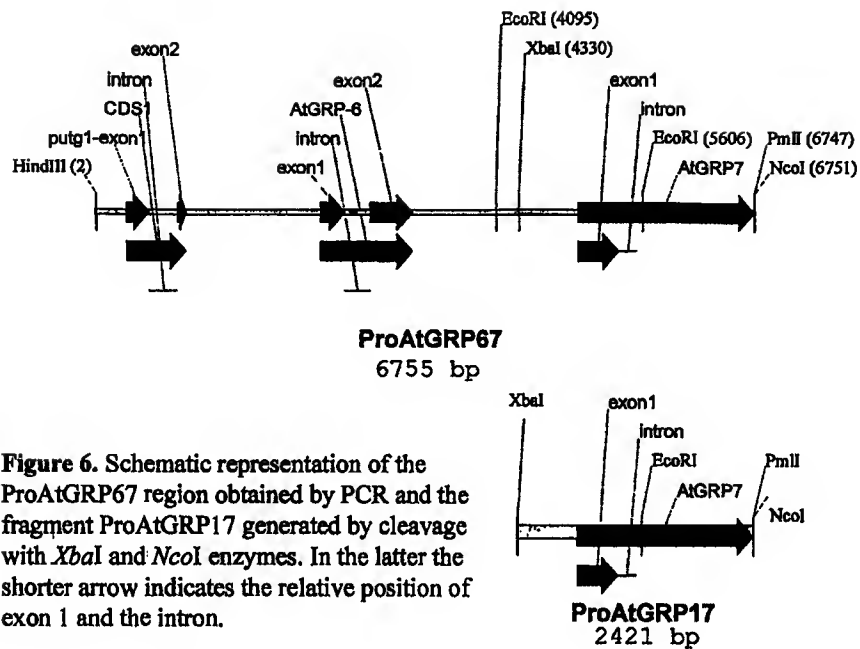


Figure 6. Schematic representation of the ProAtGRP67 region obtained by PCR and the fragment ProAtGRP17 generated by cleavage with *XbaI* and *NcoI* enzymes. In the latter the shorter arrow indicates the relative position of exon 1 and the intron.

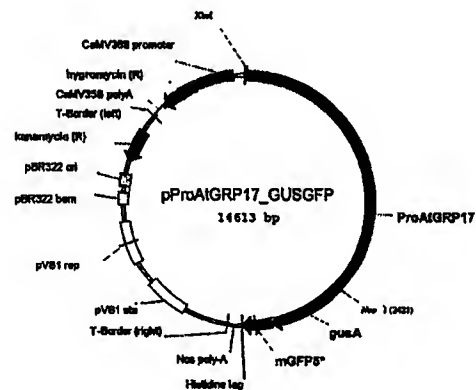


Figure 7. Schematic representation of plasmid pProAtGRP17_GUSGFP, resulting from the cloning of the PCR product ProAtGRP67 cleaved with *XbaI* and *NcoI* in the plasmid pCambia1303 cleaved with the same enzymes. The 616 bp region corresponding to part of the AtGRP17 promoter region and the AtGRP17 gene ORF are indicated as ProAtGRP17. Reporter genes GUS and GFP are also indicated.



Figure 8 – 1% agarose gel containing the expected DNA fragments of the plasmids extracted from transformed *E. coli* XL1, obtained after cleavage with the indicated enzymes. 1, pCambiaProAtGRP17 *PvuII*; 2, pCambiaProAtGRP17 *BglII*; 3, pCambia *PvuII*; 4, pCambia *BglII*; 5, 1kb ladder marker.

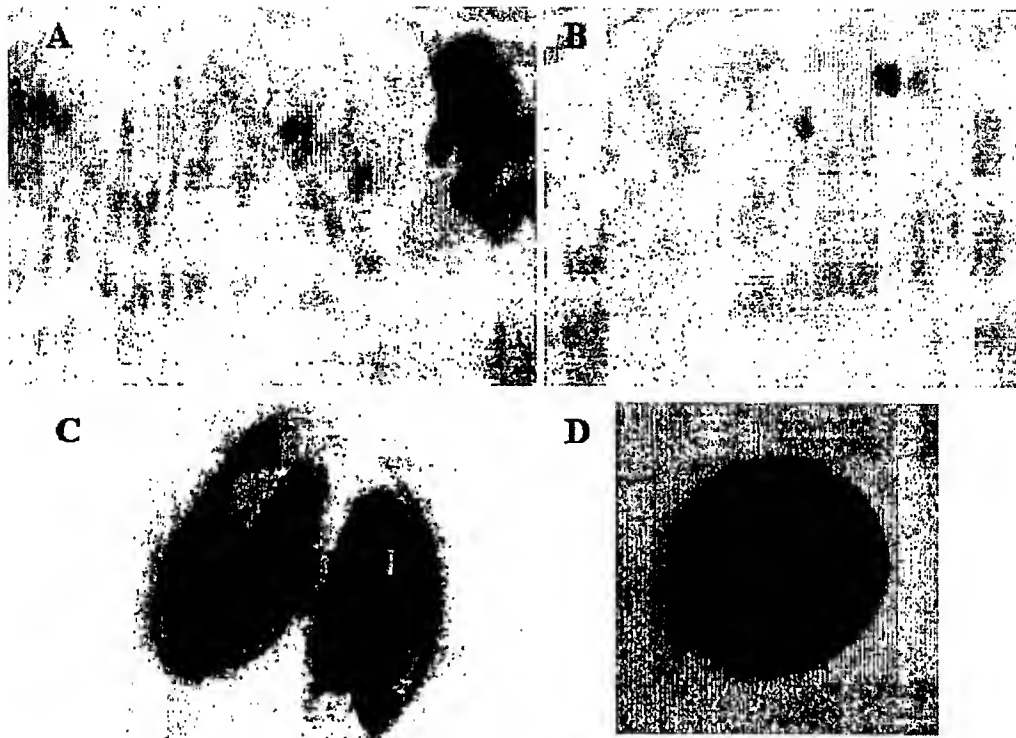


Figure 9 – Floral structures of *A. thaliana* transformed with the plasmid pCambiaProAtGRP17GUSGFP. Panel A) shows the presence and activity of GUS on the late anthers' development, but not in the initial stages of development. Panel B) shows inflorescences of the same plant in which the activity of GUS can be seen in the anthers of immature flowers (*left*) and in the anthers and petals of the mature flower (*right*). Panel C) shows intense GUS activity on the tapetum and on the pollen grains. Panel D) shows a pollen grain with positive stain for GUS. All photographs were taken under an optical microscope.

Sequence Listing

Applicant Data:

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Title of the Invention: *PHARMACEUTICAL PRODUCT AND
PRODUCTION PROCESS THEREFOR*

Number of listed sequences : 2

Seq. nr. 1

Size: 1629 base pairs

Type: cDNA

Name of the gene: AtGRP17

Function: oleosin-type protein

SEQ 1: Complete sequence of the coding region of the AtGRP17 (4940-5358) + (5545-6757)

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          a tgagcgaaga actaagtcaa aagccatcat cagctcagtc
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agagagaggg
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6661 gtccgggagt ggagggggta aacacaaaat cggaggaggt aaacacaaat
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Protein sequence translated from AtGRP17

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Seq. nr. 2

Size: 1658 base pairs

Type: DNA

Name of the promoter: ProAtGRP17

Function: promoter

SEQ 2: Complete sequence of the promoter region of the AtGRP17 (3371-4939)

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      3481 tatgtgaatg attcaatcgt gagacattga aattgtcgtt tctccattac
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